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Familial Recurrence of *SOX2* Anophthalmia Syndrome: Phenotypically Normal Mother with Two Affected Daughters

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Abstract

The *SOX2* anophthalmia syndrome is emerging as a clinically recognizable disorder that has been identified in 10–15% of individuals with bilateral anophthalmia. Extra-ocular anomalies are common. The majority of *SOX2* mutations identified appear to arise *de novo* in probands ascertained through the presence of anophthalmia or microphthalmia. In this report, we describe two sisters with bilateral anophthalmia/microphthalmia, brain anomalies and a novel heterozygous *SOX2* gene single-base pair nucleotide deletion, c.551delC, which predicts p.Pro184ArgfsX19. The hypothetical protein product is predicted to lead to haploinsufficient *SOX2* function. Mosaicism for this mutation in the *SOX2* gene was also identified in their clinically unaffected mother in peripheral blood DNA. Thus it cannot be assumed that all *SOX2* mutations in individuals with anophthalmia /microphthalmia are *de novo*. Testing of parents is indicated when a *SOX2* mutation is identified in a proband.

Keywords

anophthalmia; microphthalmia; *SOX2* anophthalmia syndrome

INTRODUCTION

The *SOX2* anophthalmia syndrome is emerging as a recognizable pleiotropic phenotype. In screening groups of individuals with anophthalmia or microphthalmia, *SOX2* mutations account for 10–15%. [Ragge et al., 2005; Faivre et al., 2006; Williamson et al., 2006]. Extra-ocular anomalies are common. The phenotype includes bilateral anophthalmia or severe microphthalmia, variable developmental delay with specific motor abnormalities, seizures,

brain anomalies, male genital anomalies, mild dysmorphic facial features and short stature [Ragge et al., 2005].

To date most individuals described with *SOX2* anophthalmia syndrome have had *de novo* mutations in *SOX2* with bilateral anophthalmia or microphthalmia as the presenting feature. *SOX2* mutations have also been identified in a group of individuals with anophthalmiaesophageal-genital (AEG) syndrome (OMIM 600992), an association of anophthalmia or microphthalmia, esophageal atresia with or without tracheo-esophageal fistula, and urogenital anomalies, most commonly cryptorchidism, hypospadias and micropenis [Williamson et al., 2006]. Recently Zenteno et al. [2006] described discordant monozygotic twin males who have c.70del20 mutation in *SOX2*. One twin had unilateral anophthalmia and esophageal atresia and the other twin had normal size corneas but a short palpebral fissure of one eye and esophageal atresia. All other studies were unremarkable. Chassaing et al. [2007] proposed germinal mosaicism in the mother of two affected female pregnancies with markedly different phenotypes. The first child had AEG syndrome with type III esophageal atresia. Prenatal ultrasound in the second pregnancy noted progressive hydrocephalus. Autopsy of the female fetus showed brain anomalies and 11 pairs of ribs but normal eyes including normal ocular microscopy. Both offspring had a c.70_86del mutation, which was not found in either parent on sequencing.

We describe two sisters with bilateral anophthalmia, brain anomalies and a novel heterozygous c.551delC *SOX2* mutation, which predicts p.Pro184ArgfsX19.

CLINICAL REPORT

The proband (Fig 1) was born at term to a G3P2 32-year-old healthy mother. Parents are not consanguineous and have two unaffected older children. This 7-year-old girl had right anophthalmia and left marked microphthalmia with light perception in the left eye only. Prenatal ultrasound showed a right arachnoid cyst and mild hydrocephalus. The patient's Apgar scores were 8 and 9. Her birth weight was 3685 g (~50th centile) and birth length was reportedly normal. A cranial MRI at age 3 years showed hypoplastic globes, right middle fossa arachnoid cyst, mild hydrocephalus, and partial absence of the posterior aspect of the corpus callosum including the splenium. Electroencephalogram confirmed partial complex seizures at 3 years of age. A sleep study confirmed disordered breathing with obstructive and central hypopnea.

Feeding and swallowing difficulties were present up to age 5 years. Growth hormone deficiency was diagnosed at age 20 months. Her IGF Binding Protein was 0.8 mg/L. She responded to human growth hormone therapy. At 19 months she had a TSH level of 3.9 uIU/mL; normal range (0.4–2.5 uIU/mL) and thyroid hormone supplements were initiated.

The patient had speech delays with receptive language better than expressive. She used limited sign language but was essentially non-verbal. She recognized family members and showed affection. She had motor delays and walked at age 4 years with assistance. She had a wide-based ataxic gait and locked her knees for balance. She needed her hand held or a walker for stability outside the house. Her mother reported that her daughter continued to make developmental progress in all areas except speech. She was not toilet trained.

On examination at age 7 years she weighed 19 kg (~25th centile); her height was 109.2 cm (<3rd centile) and her OFC was 48.2 cm (3rd to 5th centile). Bilateral clinical anophthalmia with deep set eyes and facial asymmetry were noted. She had a prominent down curved nasal tip, small, widely spaced teeth, diastasis recti, and hypoplastic labia minora. She had tactile defensiveness.

The second affected child in this family was identified prenatally. An ultrasound at 23 weeks gestation revealed underdevelopment of the globes (Fig 2). Anophthalmia was suspected based on the family history of an affected sibling. An amniocentesis was performed and chromosome study and the karyotype was 46,XX. Her birth weight was 4054 g and length was 52 cm (both appropriate for gestational age). Newborn hearing screen was normal. On ultrasound of the orbit shortly after birth, no globe was noted bilaterally. She had no response to light.

When re-examined at 4 months her height was 61.2cm (~50th centile), her weight was 5.3kg (~25th centile) and OFC was 37.8cm (<3rd centile). No dysmorphic features were described except for absent ocular structures and short palpebral fissures. (Fig 1). A cranial MRI at age 4 months showed partial agenesis of the corpus callosum and hypoplasia of the orbits and maxillae with visualization of the extraocular muscles and lacrimal glands. Minimal ocular tissue was noted in the orbit. The optic nerves were not visualized.

At about 9 months she had a TSH level of 10.5 uIU/mL; normal range (0.4–2.4 uIU/mL) and was treated with thyroid supplements. She was growing along a low centile but growth hormone levels were normal.

At age 14 months she developed blotchy red areas of skin on her back and abdomen. These regressed but have been replaced with hyperpigmented areas and were diagnosed as urticaria pigmentosa.

Her development was delayed. At age 16 months she sat without support, rolled, babbled with consonants and vowels. She finger fed herself and had no feeding difficulty. Her development was more advanced than that of her sister, the proband, at the equivalent age.

Head circumference at 25 months was 43.3 cm (< 3rd centile). She was interactive, babbling and using occasional words like “Mama” and Dada”. She could feed herself and transfer toys from hand to hand. Muscle tone was generally decreased but she was able to bear weight although she could not walk.

Unaffected mother

The mother (Fig 1) had normal vision and an unremarkable ophthalmology examination. She had normal motor abilities and apparently normal intellect. Cranial MRI was normal.

METHODS

Clinical data were collected by the Anophthalmia/Microphthalmia Registry at the Albert Einstein Medical Center by review of medical records and clinical evaluation of the proband by one of the authors (AS). Institutional Review Board (IRB) approval has been obtained from the AEMC IRB. This study was also approved by the IRB of the Children's Hospital of Philadelphia. Peripheral blood samples (10–20 ml) and buccal swabs were collected and genomic DNA was extracted using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Cord blood was collected from the affected sibling at birth for DNA extraction and testing. Standard PCR was performed on genomic DNA of affected patients, unaffected relatives, and healthy controls (matched for race and ethnicity) to amplify the 11 primer pair sets designed to cover *SOX2* exonic regions. An additional 120 base pairs of intronic sequence at both the 5'- and 3'- ends were also analyzed. Patient DNA samples were first amplified and screened for possible mutations using conformation sensitive gel electrophoresis [Ganguly, 2002, Korkko et al., 1998].

Samples with abnormal migration patterns were selected for direct sequencing. These samples were purified by ExoSap-IT (USB Corp., Cleveland, OH) per manufacturer's protocol. Direct sequencing was performed on the 3730 DNA Analyzer (Applied Biosystems, Inc.) using the Big Dye Termination v3.1 Cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA). All migration pattern changes underwent a second screening using bi-directional sequencing of patient DNA samples, with comparative normal controls.

To establish maternal mosaicism PCR sequencing was performed with the 3' *SOX2* primers (5'GGCGTGAACCAGCGCATGG and 5'GGAGCGTACCGGGTTTTCTC) as previously described [Williamson et al., 2006] on amplicons generated from genomic DNA from the mother and both affected children.

RESULTS

A novel heterozygous *SOX2* c.551delC mutation was noted in the proband and her sibling. The identical mutation was identified in the unaffected mother on sequencing of the peripheral blood with an apparently lower level of mutant allele than the probands. Maternal grandparents were tested at GeneDx laboratories (Gaithersburg, MD) for this familial *SOX2* mutation and neither carried the mutation.

This mutation predicts p.Pro184ArgfsX19. We hypothesize that the predicted shortened protein product results in loss of *SOX2* function.

DISCUSSION

Anophthalmia and microphthalmia (A/M) are considered to be the severe end of a spectrum of ocular anomalies, which include coloboma. These malformations are etiologically heterogeneous. Recent studies have demonstrated that heterozygous loss of function mutations in *SOX2* in 10–15% of patients with bilateral anophthalmia [Faivre et al., 2006; Williamson et al., 2006]. Most of the mutations are *de novo* in the probands.

We report the third family with an unaffected mother who has two offspring with bilateral A/M and a mutation in *SOX2* [Faivre et al., 2006; Chassaing et al., 2007]. All the families described to date have two female children. Of interest is that the mutation in the mother in the family described by Faivre et al. [2006] was identified with lower intensity in peripheral blood and buccal studies compared to the probands and, like the present case, was considered to be a consequence of somatic mosaicism.

Chassaing et al. [2007] proposed germinal mosaicism in the mother of two affected female pregnancies with markedly different phenotypes. In the second pregnancy, terminated at 20 weeks, the fetus had normal eyes and significant brain anomalies with a non-mosaic *SOX2* deletion mutation, which had been identified in the sibling with bilateral anophthalmia. The deletion was not identified in the peripheral blood or buccal cells of the mother on sequencing. Semiquantitative capillary electrophoresis of fluorescent *SOX2* PCR fragments, encompassing the deleted region, detected mosaicism of approximately 3% for the *SOX2* deletion identified in her children.

The frame-shift mutation described in this report is predicted to cause a premature termination of the coding sequence. Two tissues were studied in all individuals; peripheral blood DNA was initially studied and the confirmation used buccal cell DNA. There was evidence of reduced signal in the unaffected mother thus confirming somatic mosaicism. However, the level of mosaicism can not be accurately quantified (Fig 3).

Common to these three families, are maternal transmission and affected female children. Ragge et al. [2005] noted that there was an excess of females in her cohort and that males appear to be more severely affected. Some males with *SOX2* anophthalmia syndrome have genital tract anomalies [Ragge et al., 2005; Williamson et al., 2006]. Interestingly, the heterozygous *Sox2* knockout mouse shows reduced male fertility as its only anomaly [Avilion et al., 2003]. This may reflect a sex influenced effect of *SOX2* mutation. There is also no indication of imprinting in this area of chromosome 3q to explain the preponderance of females affected with *SOX2* anophthalmia. [Xiao et al., 2006].

A recent report identified monozygotic twin brothers with a *SOX2* 20 base pair deletion and markedly discordant ocular phenotypes [Zenteno et al., 2006]. Twin A had unilateral anophthalmia and esophageal atresia. Twin B had esophageal atresia with normal ophthalmologic evaluation apart from a narrow palpebral fissure on the right. These cases and the family we report here provide evidence that significant levels of mosaicism for a *SOX2* loss-of function mutation can be found in individuals with normal ocular phenotype confirming previous work that shows not all cases are the result of a new mutation. The phenotype of *SOX2* anophthalmia syndrome is variable even with the same mutation, as underscored by the twins described above and the description of Chassaing et al. [2007].

Developmental delay is common with delayed motor milestones and an unsteady ataxic gait. The family described here demonstrates that differences in development may occur in siblings with the same *SOX2* mutation. Hypothyroidism has not been described previously in *SOX2* anophthalmia syndrome. Given, the finding of hypothyroidism in these children, clinicians who evaluate patients with *SOX2* anophthalmia syndrome should consider screening for hypothyroidism if clinically indicated. The urticaria pigmentosa in the sibling of the proband may be unrelated to the *SOX2* mutation. Some cases of urticaria pigmentosa have been identified in families as autosomal dominant with incomplete penetrance. However most cases of pediatric mastocytosis in children, of which urticaria pigmentosa is the most frequent variant, are sporadic. [Rosbotham et al., 1999; Ben-Amitai et al., 2005] As more cases of *SOX2* anophthalmia syndrome are identified the phenotype will likely expand.

In conclusion, the finding of an unaffected parent with a mosaic *SOX2* mutation raises issues about the evaluation of the parents of all individuals with anophthalmia. We recommend *SOX2* mutation testing for any patient with anophthalmia or microphthalmia. A positive result should include testing the parents for the specific *SOX2* mutation. However, the level of mosaicism varies and may be missed on sequencing as in the case of Chassaing et al [2007]. Recurrence risk assessments in these families should take into account potential germinal mosaicism with low levels of somatic mosaicism. Prenatal testing in future pregnancies should be made available following the birth of a child with a *SOX2* mutation. This will enable the genetics community to provide accurate recurrence risks to these individuals and families. It will also enable prenatal or preimplantation testing for *SOX2* mutations in those families with a parent suspected or proven to be mosaic.

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Figure 1. Phenotypically unaffected mother with proband on the left and sibling on her lap. The features in the proband include bilateral anophthalmia, downturned nasal tip and small, widely spaced teeth

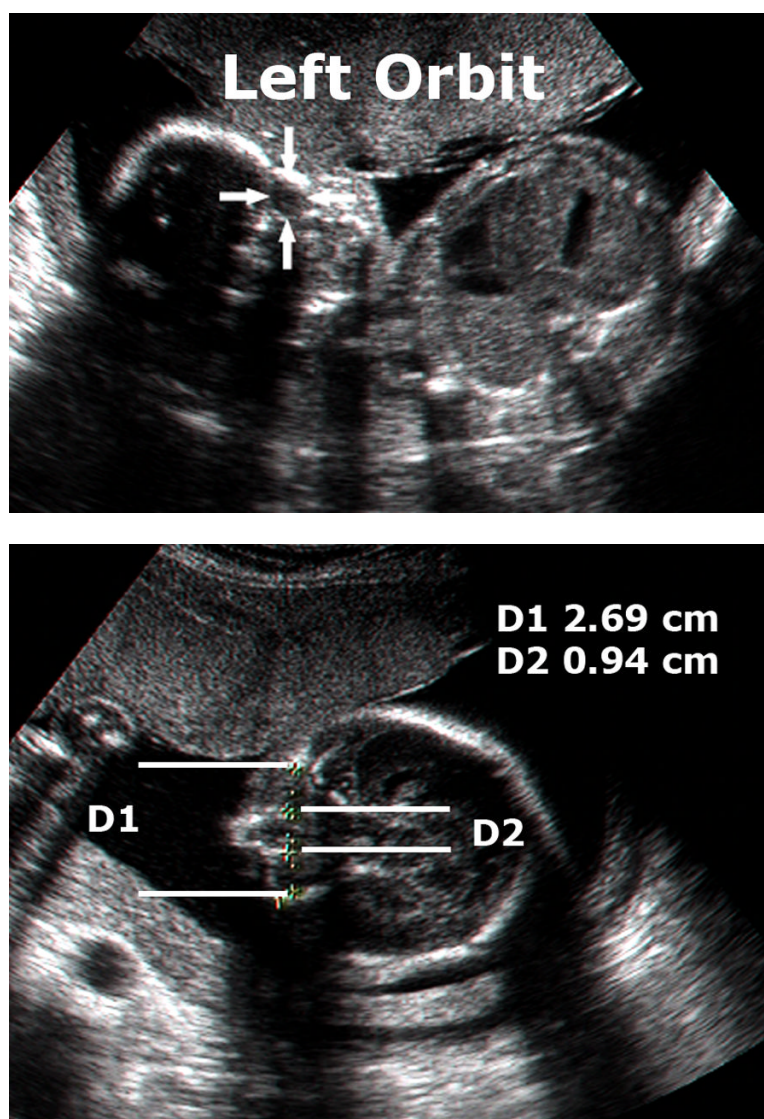


Figure 2.

Prenatal sonogram of fetus at 23 weeks gestation

A. Frontal view of left orbit shows B. Axial view through both orbits. Inner and outer orbital measurements below 5th centile for gestational age. Outer orbital distance 2.69cm Inner orbital distance 0.94cm Axial orbital measurement estimated 8 mm (below -2 SD for gestational age)

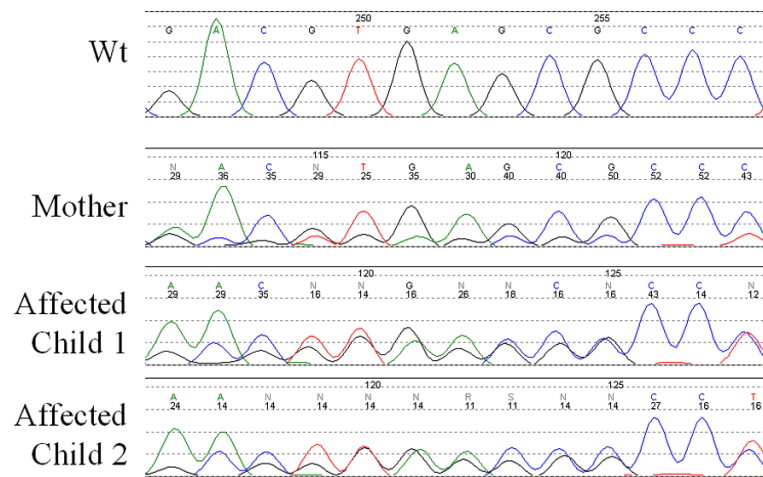


Figure 3.

Confirmation of maternal mosaicism: The c.551delC (g.982delC NC_000003 REGION: 182912416..182914918) mutation creates frameshift in the forward strand of the sequencing trace. The individual peak heights were used to calculate a wild-type to mutant ratio for the mother and both affected children. The uncorrected ratios were 3.2, 1.4 and 1.5 respectively. Normalizing the ratio by assuming the affected children are obligate heterozygotes gives a corrected ratio for the mother of 2.3 and strongly suggests that the mother is mosaic.